Environmentally induced variation in the leaf volatile terpenes of *Taxodium distichum* (L.) Rich. (Cupressaceae)

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ABSTRACT

Volatile leaf oils of *Taxodium distichum*, from two clonal lines, 478-17 and 492-23, were analyzed from test plots in Arkansas, Florida, Iowa, Kansas, and College Station and El Paso, Texas. ANOVA of oil yield and 15 terpenoids (mg/g DM) for clone 478-13 revealed one highly significant difference (bornyl acetate) and five significantly different variables (oil yield, α-pinene, terpinolene, caryophyllene oxide, and humulene epoxide II) among the test plots. Oil yield was significantly higher in the El Paso plot (10.1 mg/g) and decreased to the lowest level (7.0 mg/g) in Florida. The yields of α-pinene, bornyl acetate, caryophyllene oxide and humulene epoxide were also highest in the El Paso plot. ANOVA of 478-17 components on a % total oil basis showed a different pattern with four compounds being highly significantly different among plots (terpinolene, α-terpineol, (E)-caryphyllene, α-humulene) and two significantly different (bornyl acetate, humulene epoxide II). The major oil component, α-pinene, was not significantly different, ranging from 78.50% (College Station) to (64.43% (Arkansas). ANOVA of oil yield and 15 terpenoids (mg/g DM) for clone 492-23 revealed only one significant difference (eudesma-4(15),7-dien-1-β-ol) among the test plots, being highest in Arkansas and lowest in Iowa. ANOVA of 492-23components on a % total oil basis showed considerably greater variation with six compounds being highly significantly different among plots (α-pinene, β-pinene, (E)-caryphyllene, α-humulene, α-cadinol, eudesma-4(15),7-dien-1-β-ol) and one significantly different (germacrene D). The highest percent of α-pinene, the major component, was in Kansas (74.37%) and the lowest in Florida (59.40%). PCA did not find high correlations between growth and edaphic variables, and oil yields or oil components. Published on-line **www.phytologia.org** *Phytologia 96(3): 167-177 (July 1, 2014)*. ISSN 030319430

KEY WORDS: *Taxodium distichum*, terpenes, leaf essential oil, environmental induced differences.

Taxodium Rich. is a small genus in which one to three species have been recognized. Britton (1926), Dallimore & Jackson (1966) and Rehder (1940) all recognized three species: bald cypress, *T. distichum* (L.) Rich., pond cypress, *T. ascendens* Brongn. and Montezuma or Mexican bald cypress, *T. mucronatum* Ten. Watson (1985) treated *T. ascendens* as *T. d.* var. *imbricarium* (Nutt.) Croom. Both Farjon (2005) and Eckenwalder (2009) recognized *T. distichum*, *T. d.* var. *imbricarium* and *T. mucronatum*. Denny (2007) treated the genus as monotypic with one species, *T. distichum* and two varieties: var. *imbricarium* and var. *mexicanum* (Carr.) Gord. (= *T. mucronatum*). Denny (2007) and Denny and Arnold (2007) give a lucid discussion of the historical nomenclature of the genus. Recently, Adams et al. (2012a) examined all three putative species using DNA sequences (3547 bp) of nrDNA and three cp regions (petN-psbM, trnS-trnG, ycf-psbA) and concluded that *Taxodium* was best treated as a monotypic genus with three varieties, var. *distichum* (L.) Rich., var. *imbricarium* (Nutt.) Croom and var. *mexicanum* (Carr.) Gord.

Adams et al. (2012b) reported on geographical variation in the leaf volatile terpenes of the three varieties and reviewed the literature on *Taxodium* leaf oils. A study of seasonal variation (Adams, 2012) in the leaf oils of *T. distichum* (near Amarillo, TX) revealed that oil yield increased in new leaves (April, 3.45 mg/g DM) to May (6.64) then rapidly declined to a somewhat steady state in the summer and fall (July - Oct; 2.94 - 2.32 ns). The major component, α-pinene, exhibited no significant variation on a percent total oil basis, but did differ on a mg/g DM basis. Interestingly, all the other components, besides α-pinene, varied significantly on a percent total oil basis. There was no evidence that *Taxodium* catabolizes terpenes as energy for winter storage as found in some deciduous angiosperms.

There are no literature reports on the environmental plasticity of terpenes in *Taxodium*. The purpose of the present study is to report on environmentally induced variation in the leaf volatile terpenes of *Taxodium distichum* from two clonal lines (478-17 and 492-23), grown in various test plots ranging from Iowa to Texas to Florida.

MATERIALS AND METHODS

Plant material: *Taxodium distichum* Clones 478-17 and 492-23 were a part of a larger replicated trial (Arnold et al., 2012) encompassing 24 clones on ten sites representing north – south and east – west transects across the central and southern United States. The goal of these trials were to evaluate phenotypically elite clones which had been selected from preliminary trials conducted at three sites using seedlings of provenances collected from the central and western portion of the native range of *Taxodium distichum*. From the hundreds of seedlings grown from the provenance collections on three preliminary Texas test sites, located in Dallas, Overton, and College Station, 24 advance clonal selections were made on the basis of their aesthetic properties and subsequently pared to 16 selections based on their adventitious rooting potential during vegetative propagation. These final selections were then propagated for testing on a larger number of sites in a wider range of environments. Clones 478-17 and 492-23 were among these advanced selections. Clone 478-17 was derived from seed collected in Iberia Parish, Louisiana (29°48’0”N, 91°47’24”W), whereas clone 492-23 was derived from seed collected on the Mississippi River in Louisiana (31°33’36”N, 91°26’24”W). Three vegetatively propagated ramets of each of these clones were grown in 2.3 L containers filled with a pine bark based substrate and then shipped to each of the test sites at Ames, IA, College Station, TX, El Paso, TX, Fayetteville, AR, Haysville, KS, and Quincy, FL in spring 2009. Trees were planted in a completely randomized design on 2 m within row and at least 3 m between row spacings. Initial measurements were taken at planting and then again at the beginning and end of each growing season. Growth and aesthetic data collected during each of the three years included tree height, trunk diameter at 15 cm above the root collar, proportion of the canopy exhibiting chlorotic or necrotic foliage, winter dieback, and cold damage ratings. The tissue samples for volatile oils were derived from the trees in these plantings during the fourth growing season following planting. All leaf samples were collected between 9 and 11 am, local time, on July 16 or 17 and immediately shipped on ice for extraction. Voucher specimens are deposited in the Herbarium, Texas A & M University (for the plot materials).

Isolation of Oils - Fresh leaves (200 g), to which 2 mg, methyl decanoate (internal standard) was added to the leaves after they were placed into the circulatory Clevenger-type apparatus, were steam distillated for 2 h (Adams, 1991). Oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. Extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. Oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software and the internal standard amounts.

Data Analysis - Terpenoids (as percent total oil) were coded and compared among the taxa by the Gower metric (Gower, 1971). ANOVA and SNK (Student-Newman-Keuls') multiple range tests follows the formulation of Steel and Torrie (1960). Principle coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). Principle Components Analysis (PCA) follows the formulation of Veldman (1967).

RESULTS AND DISCUSSION

Clone 478-13

ANOVA of oil yield and 15 terpenoids (mg/g DM) for clone 478-13 revealed one highly significant difference (bornyl acetate) and five significantly different variables (oil yield, α-pinene, terpinolene, caryophyllene oxide, and humulene epoxide II) among the test plots (Table 1). Oil yield was significantly higher in the El Paso plot (10.1 mg/g) and decreased to the lowest level (7.0 mg/g) in Florida (Table 1). The yields of α-pinene, bornyl acetate, caryophyllene oxide and humulene epoxide were also highest in the El Paso plot (Table 1). Terpinolene yield was highest in the College Station plot (0.12 mg/g, Table 1).

In contrast to the mg/ g DM data, ANOVA of components on a % total oil basis showed a different pattern with four compounds being highly significantly different among plots (terpinolene, α-terpineol, (E)-caryphyllene, α-humulene) and two significantly different (bornyl acetate, humulene epoxide II) (Table 1). The major oil component, α-pinene, was not significantly different, ranging from 78.50% (College Station) to 64.43% (Arkansas).

Height and caliper measurements were largest in the Florida plot and lowest in the Kansas plot (Table 1). Percent chlorosis was greatest in Iowa and Arkansas, but chlorosis not a problem (0.0) in other plots Table 1). Die back was most apparent in College Station, Arkansas and Kansas (Table 1).

PCA (Principle Components Analysis) factoring of the correlation matrix resulted in six eigenroots before they began to asymptote. The six eigenroots accounted for 42.9, 18.3, 11.6, 9.4, 7.4 and 3.8% (93.4%) of the variance among individuals. Factor loadings (as percent variance accounted for) are shown in Table 2. Oil yield and most monoterpenes (α-pinene, myrcene, limonene, β-phellandrene) as well as germacrene D (a sesquiterpene) and bornyl acetate are highly loaded onto component 1 (Table 2). However, β-pinene was not associated with α-pinene, but lightly loaded onto several components (eigenvectors). Terpinolene and (E)-caryophyllene have over 50% of their variance on component 2.

Component 3 accounts for modest amounts of several variables, but in no case, does it explain more than 38.8% (eudesma-4(15),7-dien-1-β-ol) (Table 2). Winter low temperature was loaded on component 4 (53.4%) and die back was loaded onto component 6 (Table 2). As with component 3, no variable was strongly loaded onto component 5. Most of the growth and edaphic variables were not strongly associated with any component (Table 2).

Ordination of the 24 variables of the first three components (accounting for 72.8% of the variance) shows two major groups: oil yield, monoterpenes, and some edaphic variables (winLo, sumHi, pH, dieb); and sesquiterpenes plus ppt, %chl, hgth and calip (Fig. 1). EU47 is isolated as is the CROX-HEII group. As seen in the factor loadings, growth variables (hgth, calip) are not closely ordinated with any terpenes as in the case of winter and summer temperatures (sumHi, winLo, Fig. 1).

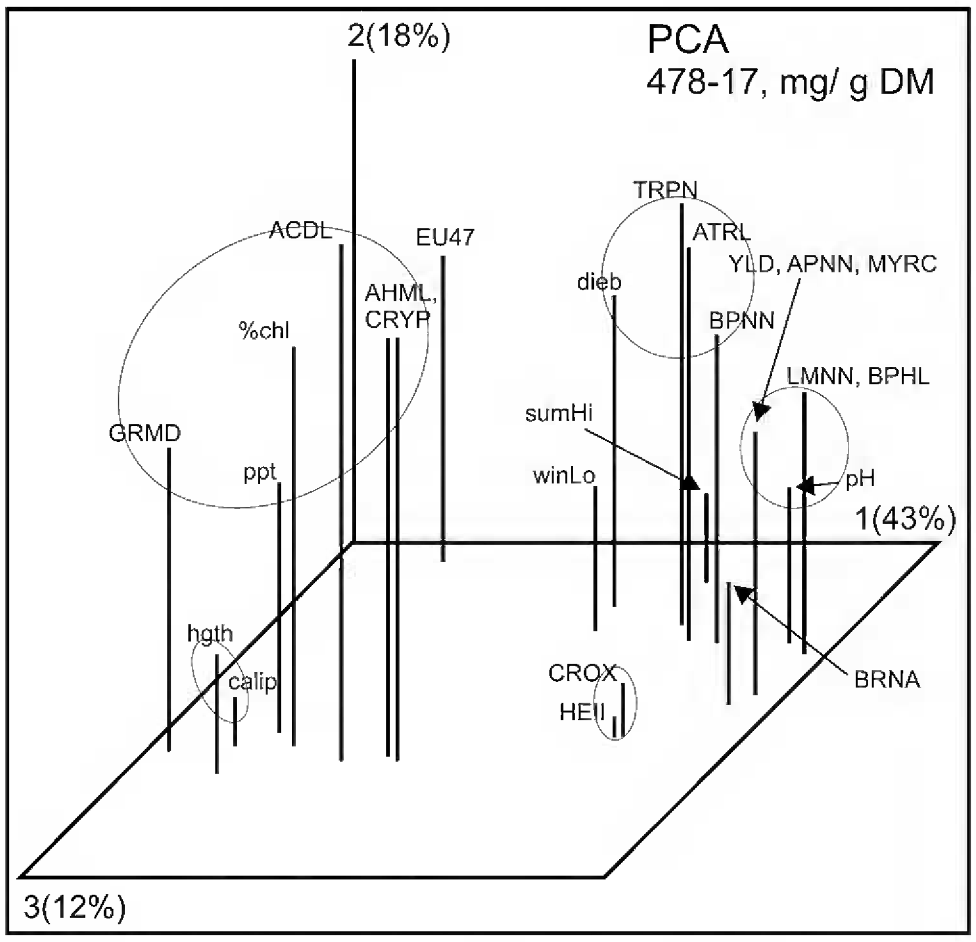

Figure 1. PCA of clone 478-17, mg/g DM basis. Circles show the major groups. See Table 1 for acronym abbreviations.

Clone 492-23

ANOVA of oil yield (mg/g DM) and 15 terpenoids for clone 492-23 revealed only one significant difference (eudesma-4(15),7-dien-1-β-ol) among the test plots, being highest in Arkansas and lowest in Iowa (Table 3). However, because most of the amounts of eudesma-4(15),7-dien-1-β-ol were very small (0.01 - 0.07), these statistics may be skewed due to the number of identical data values for this component. Oil yield was highest in the Kansas plot (7.27 mg/g) and decreased to the lowest level (4.60 mg/g) in Iowa (Table 3), but not significantly different.

As with 478-17, ANOVA of components on a % total oil basis displayed a different pattern than in the mg/ g DM data. Considerable variation was found with six compounds being highly significantly different among plots (α-pinene, β-pinene, (E)-caryphyllene, α-humulene, α-cadinol, eudesma-4(15),7-dien-1-β-ol) (Table 3) and one significantly different (germacrene D). The highest percent of α-pinene, the major component, was in highest Kansas (74.37%) and the lowest in Florida (59.40%).

PCA (Principle Components Analysis) factoring of the correlation matrix resulted in six eigenroots before they began to asymptote. The six eigenroots accounted for 39.4, 19.3, 14.4, 211.8, 5.4 and 5.2% (95.5%) of the variance among individuals (Table 4). Factor loadings (as percent variance accounted for) are shown in Table 4. Oil yield and all the monoterpenes (α-pinene, β-pinene, myrcene, limonene, β-phellandrene, terpinolene) as well as α-terpineol were highly loaded onto component 1 (Table 4). Terpinolene and (E)-caryophyllene have over 60% of their variance onto component 2.

Germacrene D and α-cadinol (sesquiterpenes) were heavily loaded onto component 3 (Table 4). Height and caliper were heavily loaded on component 4 (75.5, 57.5%, Table 4). No single variable was associated with component 5, but 82.76% of the variance in die back was on component 6 (Table 4).

Ordination of the 24 variables of the first three components accounted for 73.1% of the variance. No major groups are apparent. Minor groups are: oil yield and monoterpenes; TRPN/ATRL; CROX/HEII; AHML/CRYP; adn GRMD/ ACDL. Growth and edaphic variables (winLo, sumHi, pH, dieb) do not seem to be highly correlated with individual terpenoids (Fig. 2).

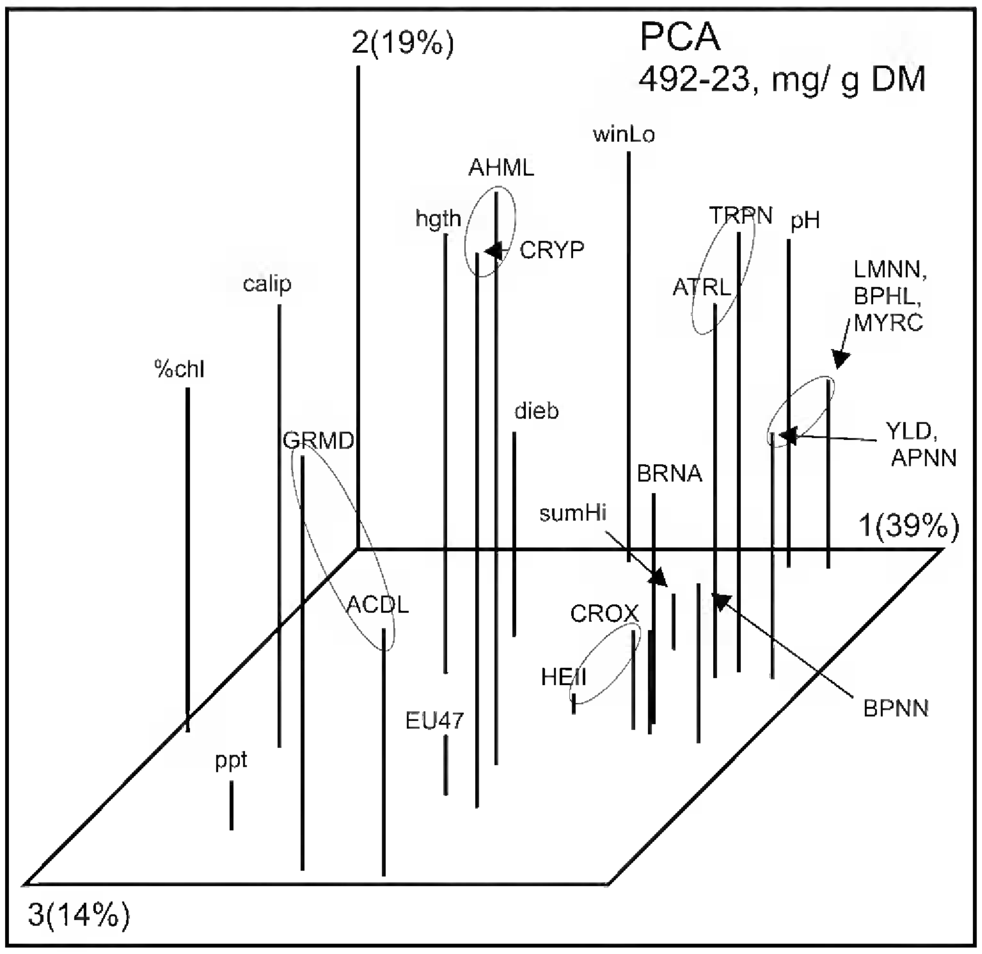

Figure 2. PCA of clone 492-23, mg/g DM basis. Circles show the major groups. See Table 2 for acronym abbreviations.

DISCUSSION

A surprising amount of variation was found in the leaf oils among clonal trees within a plot. This may be due to nature of foliage production and the large changes in terpene composition and yields in young (juvenile) and mature (adult) leaves in *Taxodium distichum*. Adams (2012), in a seasonal study, found the leaf oil increased rapidly in young leaves (April) to May (Fig. 3), then rapidly declined to July, became constant (statistically) until fall (Oct.). Three patterns of variation in the oil yield and terpenes were found: some compounds (eg. germacrene D, caryophyllene oxide, Fig. 3) increased during the summer, then declined in the fall; other compounds (caryophyllene, terpinolene, myrcene, Fig. 4) were highest in young leaves, then declined into the fall; and other terpenoid acetates and aldehyde (trans-pinocarvyl acetate, cis-pinocarvyl acetate, and germacra-4(15),5,10(14)-trien-1-al, Fig. 5) were absent

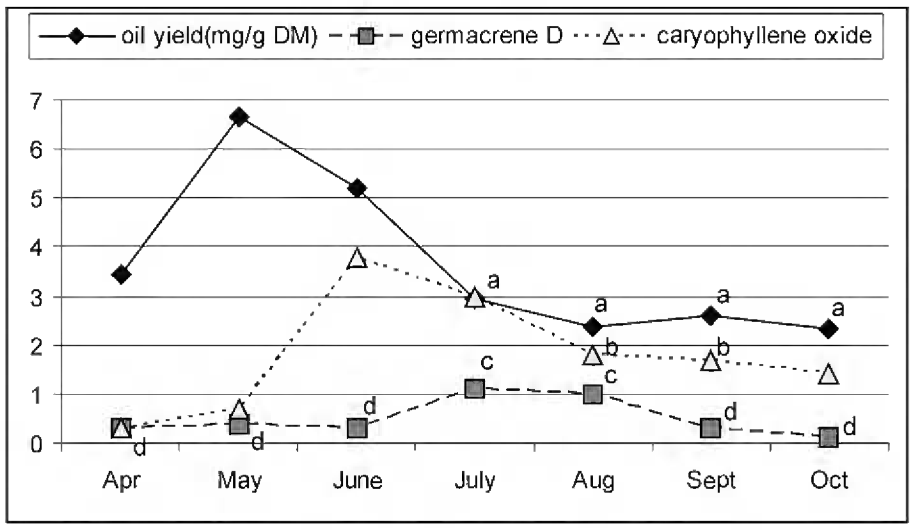

Fig. 3. Changes in oil yield, germacrene D and caryophyllene oxide during growth. (from Adams 2012).

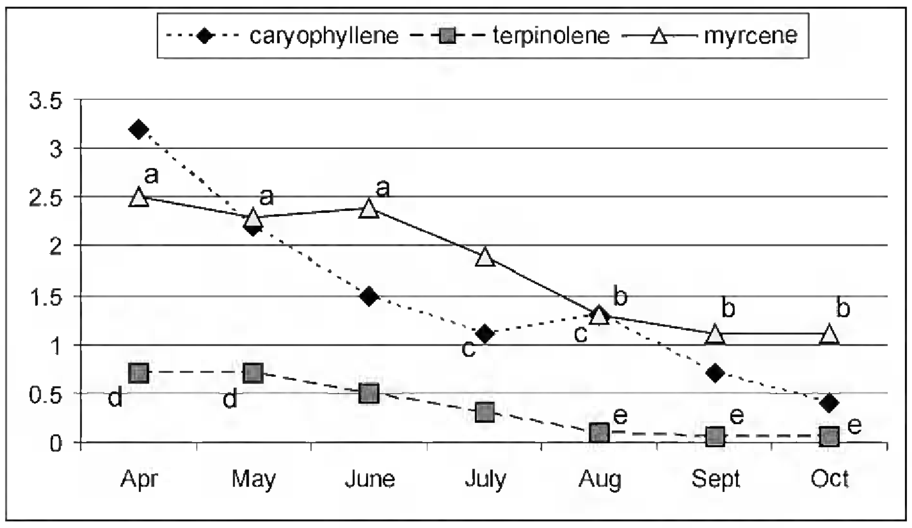

Fig. 4. Changes in caryophyllene, terpinolene, and myrcene during growth. (from Adams, 2012).

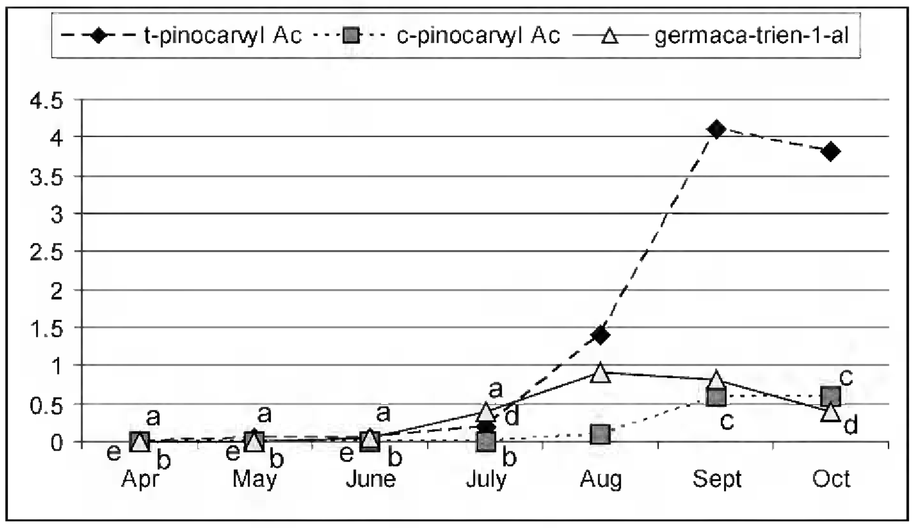

Figure 5. Changes in trans-pinocarvyl acetate, cis-pinocarvyl acetate, and germacra-trien-1-al during growth. (from Adams, 2012).

in young leaves and increased in the summer/ fall. Thus, it appears from that study, a major source of variation in oil yield and composition is differences in the ages of leaves. In the Adams (2012) study all foliage was collected from a single tree (genotype) and care was taken to remove any new flushes of leaves after the April sample (which was all young, juvenile leaves).

Thus, in the present study, even though foliage was collected on July 16 or 17, favorable growth conditions in the previous week or month, may have resulted in new flushes of foliage in some of our test plots. This likely led to a mixture of new and old leaves in the samples. This, in turn, could account for some of the variation found in the terpene composition and yields in this study.

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Table 1. Leaf essential oil composition (mg/ g DM and % total oil) for 478-17 from plots of *Taxodium distichum* in Iowa, Arkansas (Ark), Florida (FL), College Station, TX (Coll. Stat.), El Paso, TX and Kansas KS). Any number within a row with a common superscript is not significantly different (P=0.05 *). Significance = * (0.05); ** (0.01)' ns = not significant; nt = significance not tested (variable too small). Variables with highly significant differences are in boldface.

	mg/ g DM data variable	El Paso, TX	College Station, TX	Kansas	Arkansas	Iowa	Florida	signif. P =
KI	oil yield (mg/g DM)	18.10^{a}	13.20^{ab}	10.65^{ab}	8.70^{b}	8.00^{b}	7.00^{b}	0.046 *
932	α-pinene	13.75^{a}	10.32^{ab}	8.34^{ab}	5.73^{b}	6.05^{b}	5.01^{b}	0.035 *
974	β-pinene	0.22	0.25	0.15	0.11	0.15	0.10	0.137 ns
988	myrcene	0.37	0.31	0.28	0.19	0.21	0.14	0.122 ns
1024	limonene	0.17	0.14	0.16	0.09	0.08	0.06	0.148 ns
1025	β-phellandrene	0.10	0.09	0.07	0.06	0.05	0.04	0.079 ns
1086	terpinolene	0.05^{b}	0.12^{a}	0.08^{ab}	0.06^{ab}	0.08^{ab}	0.04^{b}	0.031 *
1186	α-terpineol	0.08	0.19	0.11	0.15	0.10	0.08	0.010 ns
1287	**bornyl acetate**	**0.19**^{a}	**0.10**^{b}	**0.09**^{b}	**0.11**^{b}	**0.06**^{b}	**0.06**^{b}	**0.010** **
1417	(E)-caryophyllene	0.16	0.22	0.20	0.20	0.30	0.20	0.439 ns
1452	α-humulene	0.02	0.03	0.02	0.03	0.04	0.03	0.557 ns
1480	germacrene D	0.00	0.00	0.00	0.01	0.02	0.01	nt
1582	caryophyllene oxide	0.88^{a}	0.35^{b}	0.28^{b}	0.54^{ab}	0.10^{b}	0.41^{b}	0.036 *
1608	humulene epoxide II	0.10^{a}	0.04^{b}	0.04^{b}	0.07^{ab}	0.01^{b}	0.05^{b}	0.020 *
1652	α-cadinol	0.00	0.01	0.01	0.01	0.01	0.01	nt
1687	eudesma-4(15),7-dien-1-β-ol	0.00	0.01	0.01	0.01	0.01	0.01	nt
	growth and site variables							
	height (cm)	**106.5**^{b}	**125.0**^{b}	**91.5**^{b}	**135.7**^{b}	**170.0**^{b}	**388.0**^{a}	**0.002** **
	caliper (mm)	**38.3**^{b}	**25.7**^{b}	**22.0**^{b}	**33.3**^{b}	**49.5**^{b}	**122.0**^{a}	**0.002** **
	% chlorosis	**0.0**^{b}	**0.0**^{b}	**0.0**^{b}	**3.3**^{b}	**30.0**^{a}	**0.0**^{b}	**<0.001** **
	die back (cm?)	0.0	38.3	21.5	37.7	8.5	0.0	0.598 ns
	soil pH	8.4	7.1	6.0	5.6	5.2	5.3	
	winter low tempt. (F)	3.9	26.0	5.8	-17.0	-12.0	21.0	
	annual precipitate (in.)	8.0	19.4	28.2	53.8	32.5	44.3	
	summer high tempt. (F)	108.0	109.0	109.0	114.0	96.0	105.0	
	% total oil data variable	El Paso TX	College Station, TX	Kansas	Arkansas	Iowa	Florida	signif. P =
KI	% oil yield per g DM	1.81^{a}	1.32^{ab}	1.07^{ab}	0.87^{b}	0.80^{b}	0.70^{b}	0.046 *
932	α-pinene	76.10	78.50	78.15	64.43	76.75	69.80	0.325 ns
974	β-pinene	1.20	1.93	1.40	1.23	1.90	1.57	0.363 ns
988	myrcene	2.05	2.33	2.65	2.17	2.65	2.00	0.144 ns
1024	limonene	0.93	1.02	0.95	1.04	0.93	0.83	0.278 ns
1025	β-phellandrene	0.57	0.68	0.65	0.69	0.62	0.63	0.423 ns
1086	**terpinolene**	**0.30**^{c}	**1.00**^{a}	**0.75**^{ab}	**0.70**^{b}	**1.00**^{a}	**0.63**^{b}	**0.001** **
1186	**α-terpineol**	**0.45**^{c}	**1.43**^{ab}	**1.00**^{b}	**1.77**^{a}	**1.20**^{ab}	**1.17**^{ab}	**0.007** **
1287	bornyl acetate	1.05^{ab}	0.73^{b}	0.80^{b}	1.33^{a}	0.70^{b}	0.93^{ab}	0.046 *
1417	**(E)-caryophyllene**	**0.80**^{d}	**1.63**^{c}	**1.95**^{bc}	**2.40**^{bc}	**3.75**^{a}	**2.80**^{bc}	**<0.001** **
1452	**α-humulene**	**0.09**^{d}	**0.20**^{c}	**0.25**^{bc}	**0.33**^{bc}	**0.50**^{a}	**0.37**^{bc}	**<0.001** **
1480	germacrene D	0.00	0.00	0.02	0.02	0.20	0.04	nt
1582	caryophyllene oxide	4.75^{ab}	2.47^{ab}	2.65^{ab}	6.53^{a}	1.20^{b}	6.47^{ab}	0.060 ns
1608	humulene epoxide II	0.60^{a}	0.26^{ab}	0.35^{ab}	0.80^{a}	0.09^{b}	0.73^{a}	0.030 *
1652	α-cadinol	0.00	0.02	0.05	0.04	0.16	0.06	nt
1687	eudesma-4(15),7-dien-1-β-ol	0.00	0.01	0.03	0.02	0.02	0.05	nt

Table 2. Variance extracted by six eigenroots and percent variance explained by each variable onto eigenvector from PCA of 478-17 based on gm/g DM data for 16 terpene and 8 edaphic characters in 14 individuals in 6 test plots. Character codes are used in Fig. 1. Components (eigenvectors) accounting for 50% or more of the variance of a variable are in bold face.

		Component (eigenvector)					
		1	2	3	4	5	6
	Variance by eigenvectors, % total:	42.9 %	18.3%	11.6%	9.4	7.4%	3.8%
code	mg/ g DM data set	Variance explained by each eigenvector (%)					
YLD	oil yield (mg/g DM)	**91.7**	0.9	5.5	0.2	0.0	0.3
APNN	α-pinene	**87.2**	2.6	3.1	2.1	0.1	1.4
BPNN	β-pinene	47.1	15.5	0.2	11.7	0.7	2.2
MYRC	myrcene	**80.6**	7.2	3.7	0.0	0.0	0.5
LMNN	limonene	**77.8**	2.5	0.1	0.0	0.1	0.1
BPHL	β-phellandrene	**90.7**	5.9	0.0	0.0	0.8	0.4
TRPN	terpinolene	27.9	**53.8**	3.2	0.8	10.5	0.5
ATRL	α-terpineol	21.0	28.1	8.6	11.6	19.9	5.5
BRNA	bornyl acetate	**77.4**	4.4	7.1	5.9	1.6	0.5
CRYP	(E)-caryophyllene	0.0	**55.5**	26.9	1.4	9.1	2.3
AHML	α-humulene	1.3	41.4	32.5	9.4	7.5	0.9
GRMD	germacrene D	**58.3**	11.6	22.4	0.1	2.2	0.7
CROX	caryophyllene oxide	40.7	27.5	12.7	11.1	1.2	2.9
HEII	humulene epoxide II	39.3	32.0	10.7	13.8	0.1	1.4
ACDL	α-cadinol	35.4	29.1	5.7	0.1	2.6	8.9
EU47	eudesma-4(15),7-dien-1-β-ol	33.6	9.6	38.8	0.2	0.7	4.7
hght	height (cm)	33.0	15.9	13.0	6.2	28.9	0.7
calip	caliper (mm)	24.5	21.8	20.0	7.5	20.5	0.9
%chl	% chlorosis	16.8	41.5	18.4	0.1	15.2	1.4
dieb	die back (cm?)	2.3	11.7	14.1	9.3	2.1	**53.6**
pH	soil pH	73.8	3.9	1.9	8.4	7.1	0.2
winLo	winter low tempt. (F)	4.5	1.7	5.5	**53.4**	32.5	0.1
ppt	annual precipitate (in.)	47.2	1.1	0.2	38.8	8.8	0.0
sumHi	summer high tempt. (F)	16.8	14.9	23.7	33.9	4.8	0.9

Table 3. Leaf essential oil composition (mg/ g DM and % total oil) for 492-23 from plots of *Taxodium distichum* in Iowa, Arkansas (Ark), Florida (FL), College Station, TX (Coll. Stat.), El Paso, TX and Kansas KS). Any number within a row with a common superscript is not significantly different (P=0.05 *). Variables with highly significant differences are in boldface.

	mg/g DM data variable	Kansas	College Station, TX	Arkansas	Florida	Iowa	signif. P =
KI	oil yield (mg/g DM)	7.27	6.87	6.10	5.45	4.60	0.250 ns
932	α-pinene	5.41	4.55	4.16	3.21	2.94	0.070 ns
974	β-pinene	0.13	0.12	0.14	0.09	0.10	0.342 ns
988	myrcene	0.25	0.23	0.18	0.16	0.15	0.077 ns
1024	limonene	0.07	0.07	0.06	0.05	0.04	0.070 ns
1025	β-phellandrene	0.05	0.05	0.04	0.04	0.03	0.218 ns
1086	terpinolene	0.05	0.06	0.03	0.04	0.03	0.329 ns
1186	α-terpineol	0.07	0.08	0.06	0.06	0.04	0.356 ns
1287	bornyl acetate	0.02	0.03	0.02	0.02	0.02	0.618 ns
1417	(E)-caryophyllene	0.39	0.39	0.23	0.47	0.43	0.319 ns
1452	α-humulene	0.05	0.05	0.03	0.06	0.05	0.276 ns
1480	germacrene D	0.05	0.03	0.06	0.16	0.09	0.263 ns
1582	caryophyllene oxide	0.16	0.24	0.26	0.14	0.06	0.166 ns
1608	humulene epoxide II	0.01	0.03	0.04	0.01	0.01	0.088 ns
1652	α-cadinol	0.04	0.04	0.08	0.10	0.04	0.172 ns
1687	eudesma-4(15),7-dien-1-β-ol	0.03^{a}	0.03^{ab}	0.07^{ab}	0.05^{ab}	0.01^{ab}	0.026 *
	growth and site variables						
	height (cm)	**183.0^{c}**	**244.4^{b}**	**139.3^{c}**	**309.5^{a}**	**138.3^{c}**	**<0.001 ****
	caliper (mm)	**32.7^{b}**	**54.1^{b}**	**30.0^{b}**	**103.0^{a}**	**46.3^{b}**	**<0.001 ****
	% chlorosis	**0.0^{b}**	**0.0^{b}**	**3.3^{b}**	**0.0^{b}**	**36.7^{a}**	**<0.001 ****
	die back (cm?)	0.0	38.3	37.7	0.0	1.7	0.370 ns
	soil pH	6.0	7.1	5.6	5.3	5.2	
	winter low tempt. (F)	5.8	26.0	-17.0	21.0	-12.0	
	annual precipitate (in.)	28.2	19.4	53.8	44.3	32.5	
	summer high tempt. (F)	109.0	109.0	114.0	105.0	96.0	
	% total oil data variable	Kansas	College Station, TX	Arkansas	Florida	Iowa	signif. P =
KI	% oil yield per g DM)	0.73	0.69	0.61	0.55	0.46	0.250 ns
932	**α-pinene**	**74.37^{a}**	**67.63^{ab}**	**69.23^{ab}**	**59.40^{c}**	**63.77^{bc}**	**0.007 ****
974	**β-pinene**	**1.80^{b}**	**1.87^{b}**	**2.23^{a}**	**1.70^{b}**	**2.20^{a}**	**0.001 ****
988	myrcene	3.43^{a}	3.40^{a}	3.00^{ab}	2.80^{b}	3.23^{ab}	0.056 ns
1024	limonene	0.99	1.03	0.95	0.93	0.94	0.265 ns
1025	β-phellandrene	0.68	0.70	0.65	0.62	0.63	0.220 ns
1086	terpinolene	0.67	0.83	0.53	0.60	0.77	0.235 ns
1186	α-terpineol	1.03	1.20	1.00	1.05	0.83	0.541 ns
1287	bornyl acetate	0.30	0.43	0.40	0.35	0.40	0.142 ns
1417	**(E)-caryophyllene**	**5.37^{bc}**	**5.90^{b}**	**3.97^{c}**	**8.25^{a}**	**9.37^{a}**	**<0.001 ****
1452	**α-humulene**	**0.73^{b}**	**0.80^{b}**	**0.50^{b}**	**1.05^{a}**	**1.13^{a}**	**0.002 ****
1480	germacrene D	0.73^{b}	0.53^{b}	1.00^{b}	2.35^{a}	1.83^{ab}	0.020 *
1582	caryophyllene oxide	2.13	3.73	4.07	2.70	1.33	0.171 ns
1608	humulene epoxide II	0.21	0.43	0.63	0.35	0.09	0.063 ns
1652	**α-cadinol**	**0.57^{b}**	**0.53^{b}**	**1.23^{a}**	**1.65^{a}**	**0.77^{b}**	**0.006 ****
1687	**eudesma-4(15),7-dien-1-β-ol**	**0.36^{b}**	**0.37^{b}**	**1.13^{a}**	**0.90^{a}**	**0.20^{b}**	**0.001 ****

Table 4. Variance extracted by eigenroots and percent variance explained by each variable onto eigenvector from PCA of 492-23 based on gm/g DM data for 16 terpene and 8 edaphic characters in 14 individuals in 5 test plots. Character codes are used in Fig. 2. Components (eigenvectors) accounting for 50% or more of the variance of a variable are in bold face.

		Component (eigenvector)					
		1	2	3	4	5	6
	Variance by eigenvectors, % total:	39.4 %	19.3%	14.4%	11.8	5.4%	5.2%
code	mg/ g DM data set	Variance explained by each eigenvector (%)					
YLD	oil yield (mg/g DM)	**91.8**	0.4	1.1	0.8	0.1	1.3
APNN	α-pinene	**80.4**	0.1	0.5	3.5	6.2	4.2
BPNN	β-pinene	**71.3**	9.4	4.9	9.7	0.2	1.0
MYRC	myrcene	**87.1**	2.2	1.9	4.5	0.5	1.5
LMNN	limonene	**92.4**	0.0	1.0	0.9	0.1	1.2
BPHL	β-phellandrene	**94.7**	0.2	0.0	1.2	0.2	2.0
TRPN	terpinolene	**63.7**	17.1	0.6	4.2	0.0	2.3
ATRL	α-terpineol	**69.3**	5.2	0.0	0.1	8.8	0.2
BRNA	bornyl acetate	49.1	0.9	4.0	1.1	28.4	9.8
CRYP	(E)-caryophyllene	8.4	**62.8**	20.1	2.0	1.7	2.1
AHML	α-humulene	13.7	**65.1**	11.8	0.1	0.2	2.4
GRMD	germacrene D	1.0	16.6	**77.5**	0.0	0.3	3.2
CROX	caryophyllene oxide	32.6	32.4	0.0	9.1	22.43	0.0
HEII	humulene epoxide II	19.5	**52.0**	0.4	3.9	21.9	0.2
ACDL	α-cadinol	13.1	0.9	**78.1**	5.7	0.2	0.6
EU47	eudesma-4(15),7-dien-1-β-ol	17.4	38.9	30.7	8.3	0.0	0.5
hght	height (cm)	0.8	16.8	0.4	**75.5**	0.0	3.2
calip	caliper (mm)	8.4	22.5	6.7	**57.5**	0.1	1.1
%chl	% chlorosis	38.0	3.5	2.5	36.8	11.0	0.4
dieb	die back (cm?)	0.3	3.7	6.9	0.8	2.7	**82.76**
pH	soil pH	40.2	2.0	46.2	3.3	1.6	2.6
winLo	winter low tempt. (F)	14.8	32.9	9.1	40.2	1.6	0.0
ppt	annual precipitate (in.)	6.8	42.0	37.9	1.5	8.9	0.5
sumHi	summer high tempt. (F)	31.2	34.6	3.4	12.7	13.1	1.8